# Cyclosporin A

## INHIBITION OF EXPERIMENTAL AUTOIMMUNE UVEITIS IN LEWIS RATS

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ABSTRACT  Cyclosporin A (CS-A), a selective inhibitor of T lymphocytes, is reported here to prevent S antigen (S-Ag) induced uveitis in Lewis rats. The S-Ag, found in all mammalian retinas, is uveitogenic under experimental conditions and patients with certain uveitic entities demonstrate cell mediated responses to this antigen. Daily treatment with CS-A (10 mg/kg) begun on the same day as S-Ag immunization totally inhibited the development of the uveitis in this experimental autoimmune model. Moreover a greater CS-A dose (40 mg/kg) efficiently prevented the disease process when therapy was started 7 d after S-Ag immunization. Anti-S-Ag antibody titers were observed to be similar in rats either protected or not protected with CS-A. Our data support strongly the need for T cell participation in this disease model. Since ocular inflammatory disease is an important cause of visual impairment, the data further suggest that CS-A may be useful in the treatment of patients with intractable uveitis.

## INTRODUCTION

Cyclosporin A (CS-A),[1] an endecapeptide obtained from several fungal extracts, appears to be an essentially T cell-specific drug (1). It has been found to be beneficial in preventing or modulating experimental allergic encephalomyelitis, Freund's adjuvant arthritis, graft vs. host disease, reactions related to allogeneic bone marrow transplantation, and allograft rejection (2–6). The retinal S antigen (S-Ag), a protein purified from mammalian retinas (7), consistently induces with one immunizing injection, remote from the globe, a severe anterior and posterior uveitis in lower mammals and in subhuman primates (7–9). Also, some patients with posterior uveitic conditions demonstrate in vitro cell-mediated responses to this antigen (10), thus suggesting that it may play a role in the pathogenesis of their disease. We report here the successful prevention of autoimmune uveitis in rats with CS-A. In addition, the immune responses monitored during this study provide information about cellular mechanisms in this model.

## METHODS

Female Lewis rats (MA Bioproducts, Walkersville, Md.) weighing about 200 g, were immunized with a total of 30 μg of bovine S-Ag, prepared as outlined elsewhere (7). The S-Ag was emulsified 1:1 in complete Freund's adjuvant (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) enriched with the addition of 2.5 mg/ml of *M. tuberculosis* H37 RA (Difco Laboratories, Detroit, Mich.). The emulsion was injected into both hind foot pads. All animals were sacrificed 14 d after immunization. CS-A, kindly supplied by Sandoz, Ltd., was dissolved in pure olive oil, and

[1] *Abbreviations used in this paper:* CS-A, cyclosporin A; S-Ag, S antigen.

test rats received S-Ag combined with either daily subcutaneous CS-A or pure olive oil injections into the hind quarters for 14 d starting on the day of S-Ag immunization, while others received CS-A starting on day 7 after immunization and continued daily until day 14.

*Cell culture and antibody evaluation.* A serum sample was taken by cardiac puncture, and popliteal draining lymph nodes from the site of S-Ag immunization were removed at the time of sacrifice. The lymph nodes were teased, the cells washed, and lymphocyte cultures prepared as described elsewhere (11). Briefly, cultures were performed in triplicate in flat bottom Microtest II plates (Falcon Labware, Div of Becton, Dickinson & Co., Oxnard, Calif. $3 \times 10^5$ cells in 0.2 ml/well) in RPMI 1640 medium (MA Bioproducts) supplemented with 2 mM glutamine, 100 U penicillin/ml and 100 $\mu$g streptomycin/ml (Gibco Laboratories) and 10% heat-inactivated AB+ human serum. Cultures were stimulated with S-Ag, purified protein derivative (Connaught Laboratories, Toronto, Canada) and concanavalin A (Con A, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.). Tritiated thymidine $1\mu$Ci/well, (Amersham Corp., Arlington Heights, Ill.) was added for the last 16 h of culture, which were terminated 4 d after initiation. The uptake of thymidine was measured by scintillation counting, and the results for each group are presented as the stimulation index (calculated by dividing the counts per minute [cpm] of stimulated wells by the cpm of wells containing no additive) ±SE.

Determination of the anti-S-Ag antibodies by the enzyme-linked immunoassay technique was performed as reported by Rennard et al. (12). Briefly, polystyrene plate wells (Linbro Chemical Co., Hamden, Conn.) were coated by incubation with 120 ng of S-Ag for 2 h. The reactants were added in the following order: (*a*) test rat sera were serially diluted and incubated 2 h. (*b*) The peroxidase conjugated anti-rat IgG (Miles Laboratories Inc., Elkhart, Ind.) at a 1:1,000 dilution was incubated for 1 h. (*c*) The substrate for the bound enzyme was also incubated for 1 h. Incubations were at 37°C in a final volume of 100 $\mu$l. The wells were washed three times after each incubation with phosphate-buffered saline containing 0.05% Tween-20. Positive color changes were visually recorded (double blind) and compared to rat sera which were previously determined as containing or not containing anti-S-Ag antibody. The reported titers are an average of at least four separate sera from each treatment group.

*Histologic preparation and grading of inflammation.* Eyes were fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin. Histopathology was evaluated by Dr. Rodrigues without information about the protocol for treatment. The grade of inflammation, as outlined in reference 7, are averages based on eyes that developed uveitis.

## RESULTS

Control rats immunized with S-Ag and given daily injections of olive oil manifested severe ocular disease by 14 d. The globes were proptotic, and an hypopyon and/or hemorrhage was evident in the anterior chamber. Histologically, a panophthalmitis was apparent. A severe anterior chamber reaction was present with fibrin as well as acute and chronic inflammatory cells. The posterior portion of the eye showed a marked mononuclear infiltration of the retina, often destroying its normal architecture (Fig. 1). Grading the uveitis from none (0) to severe anterior and posterior disease
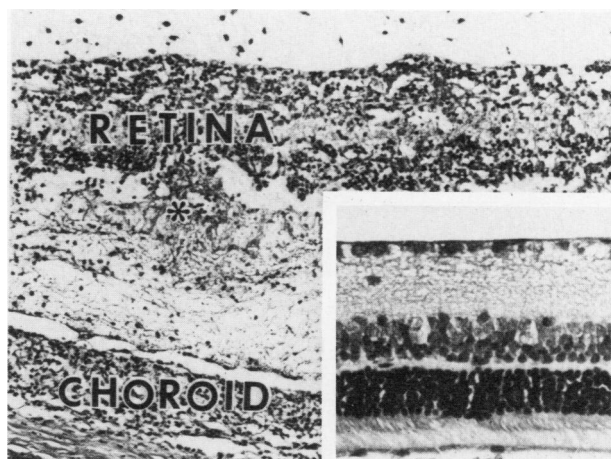


FIGURE 1  2 wk after immunization with 30 $\mu$g S-Ag marked inflammation and disorganization of the retina and choroid is present. The subretinal space between the retina and choroid contains fibrinous material and inflammatory; cells. (hematoxolin and eosin, ×130) Inset shows normal retina of the Lewis Rat (×330).

(4), the eyes taken from these animals were found to have an average grade 3.3 uveitis. However, the uveitis produced by S-Ag was markedly reduced in rats with CS-A (Table I). A complete prevention of disease was achieved by treating the rats daily with 10 mg/kg. A partial prevention, with a reduced severity in affected animals, was found in rats treated with 5 mg/kg per d of CS-A, while no clear effects were produced by 0.5 mg/kg per d of the drug. Control rats immunized with CFA alone, with or without the tested doses of CS-A manifested no ocular alterations that could be demonstrated either clinically or histologically.

All experimental groups demonstrated good proliferative responses to concanavalin A, indicating good culture viability and responsiveness. All the groups exhibited variations in response, with no statistically significant differences apparent. However, the proliferative responses to the S-Ag were significantly greater in rats treated with 0.5 or 5 mg/kg per d ($P$ <0.05), as compared to S-Ag immunized non-CS-A treated rats. The proliferative responses to purified protein derivative appeared to be depressed in rats treated with 10 mg/kg per d, but statistical analysis did not support that impression ($P \geq 0.05$).

Circulating anti-S-Ag antibodies (IgG) were found 14 d after S-Ag immunization. Sera from CS-A treated S-Ag immunized animals with no evidence of ocular inflammation had antibody titers similar to the unprotected group with uveitis (Table I).

Treatment with CS-A was also found effective even when daily injections were started 7 d after immunization with the S-Ag. However, a higher dose of 40 mg/kg

TABLE I
Effects of CS-A on Responses of S-Ag Immunized Lewis Rats

| CS-A daily injections | Number of eyes‡ | Pathology | | Stimulant in lymphocyte culture* | | |
|---|---|---|---|---|---|---|
| | | Uveitis | Grade of inflammation† | Purified protein derivative (10 µg/ml) | S-Ag (3 µg/ml) | Antibody titer |
| mg/kg | | % | | | | |
| None (olive oil) | 22 | 100 | 3.3 | 14.8±4.5 | 9.6±2.9 | 1:1,000 |
| 0.5 | 8 | 100 | 3.6 | 19.5±3.2 | 27.9±6.3§ | 1:2,750 |
| 5.0 | 8 | 25 | 2.5 | 18.2±4.2 | 48.5±18.6§ | 1:1,100 |
| 10.0 | 12 | 0 | 0 | 2.8±0.8 | 4.7±1.6 | 1:1,208 |
| 40.0‖ | 12 | 0 | 0 | 2.0±1.0 | 9.9±2.6 | 1:1,830 |

* Stimulation index±SE.
‡ Mean value of only uveitic eyes in each group.
§ $P < 0.05$ when compared to "none" group.
‖ CS-A treatment begun 7 d after S-Ag immunization. Mitotic responses reported are the result of pooled lymph node cells. Enzyme-linked immunoassay determination is the average of six individual sera.

was needed to completely suppress the uveitis in all six animals by this schedule as compared to the 10 mg/kg of CS-A needed when treatment was initiated on the day of immunization. Antibody titers were the same as the other groups reported (Table I). The draining lymph nodes from this group were very small, and did not individually yield sufficient cell numbers for culturing. However, in two experiments with pooled lymphocytes from three animals each, cells proliferated in the presence of the S-Ag. We did not observe a decrease in weight or any other toxic signs in animals receiving this relatively high dosage of CS-A.

## DISCUSSION

CS-A has been shown to be an effective mode of therapy for problems arising in transplantation immunity. The beneficial effects of CS-A in presumed autoimmune conditions have yet to be fully determined. However this reported demonstration of the effective inhibition by CS-A of this autoimmune ocular model appears to broaden the clinical and experimental application of this drug. These findings are especially relevant for at least two major reasons. The first is that this uveitis model is induced with an antigen to which some patients with posterior uveitis have shown immunological response; and the second, CS-A effectively protects animals even when therapy was begun 7 d after immunization. These conditions would simulate more the clinical problem that the ophthalmologist faces when seeing a patient with active uveitis.

The increased cell proliferation in culture to the S-Ag of lymphocytes from CS-A treated animals as compared to controls deserves comment. In groups with stimulation indexes greater than the control value, animals with and without uveitis manifested this response. Additional kinetic experiments have tentatively shown that maximum cell-mediated responses from lymph nodes of animals treated with 5 mg/kg of CS-A appear to peak about 14 d after S-Ag immunization. Peak cell-mediated responses from non-CS-A protected animals were seen some four days earlier than the CS-A treated group. It has been suggested that CS-A may have a selectivity for certain T-cell sub-populations (13–15). The delayed response to S-Ag we have seen may be a result of CS-A's effect on T cell subgroups such as helper cells, which could be concerned with the recruitment of other immunoreactive cells. An alternative or additional possibility could be that suppressor T cells are not affected or are even augmented by CSA therapy, as has been suggested to be the case in humans (15).

The finding of similar antibody titers in unprotected and CS-A protected animals is in line with the well-established selectivity of this drug toward T cells (1), and indicates the essential role of the T cell in the pathogenesis of this disease. These data indicate further that CS-A could be a new drug in the armamentarium of medications capable of altering severe ocular inflammatory reactions, which account for a significant number of visually impaired individuals.

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